RESEARCH ON THE CHEMISTRY OF PHENOXAZINES

VII.* REACTIONS AND PROPERTIES OF RESORUFIN AND SOME OF ITS DERIVATIVES

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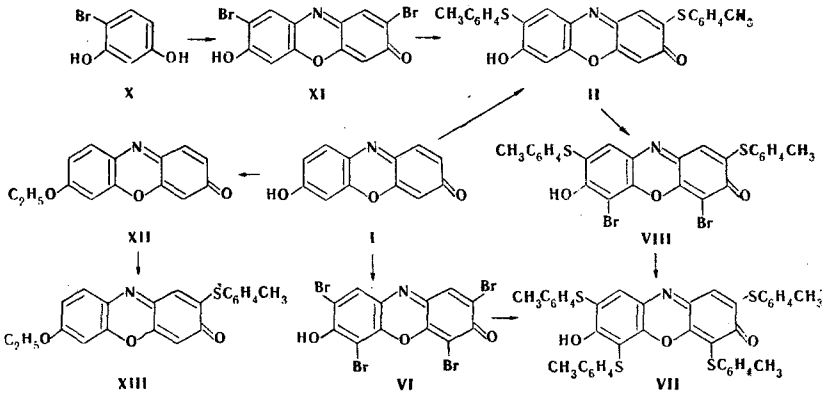
Resorufin (7-hydroxy-3-phenoxazinone) reacts with thiophenols to give 2,8-di(arylthio) derivatives. The site of entry of the nucleophilic residues indicates protonation of resorufin at the ring nitrogen atom. The changes in the absorption spectra of the arylthio derivatives of resorufin in the visible region as a function of the pH of the solution are associated with the acid-base dissociation of these compounds with respect to the hydroxyl group. The ionization constants of some 2,8- and 2,4,6,8-tetrasubstituted resorufin derivatives were measured.

3-Phenoxazinone has two electrophilic centers – one in the benzoid ring (in the para position relative to the nitrogen atom) and one in the quinoid ring [2]. In the present paper we have examined the reaction of thiophenols with resorufin (I), in which the position characteristic for nucleophilic attack in the benzoid ring is occupied by a hydroxyl group; only the reaction center in the quinoid ring remains open.

The reaction of I with thiophenols occurs only under acid catalysis conditions, i.e., under the condition of prior protonation of the resorufin molecule. In this case, despite our expectations, di(arylthio) resorufin derivatives II-V (Table 1) rather than mono(arylthio) derivatives were isolated. An examination of the resonance structures shows that activation of the 1 and 9 positions for nucleophilic attack can be expected when resorufin is protonated at the exocyclic oxygen atom. Protonation at the ring nitrogen atom will promote reaction at the 2, 4, 6, and 8 positions. The problem of protonation presently remains unsolved: thus, the addition of a proton to the carbonyl group is examined in [3, 4] (in [5] this possibility is considered for the related 3-phenothiazone), while addition of a proton to the ring nitrogen atom is considered in [6, 7].



*See [1] for communication VI.

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TABLE 1. Arylthio Derivatives of Resorufin

Compound	mp, °C	Empirical formula	Found, %				Calc., %				Yield, %
			C	H	N	S	C	H	N	S	
II	230—231[a]	$C_{26}H_{19}NO_3S_2$	68,4	4,4	3,1	14,1	68,2	4,6	3,1	14,0	23
III	280—281[a]	$C_{24}H_{15}NO_3S_2$	67,0	3,6	3,3	14,8	67,1	3,5	3,3	14,8	20
IV	288—290[a]	$C_{24}H_{13}N_3O_7S_2$	55,3	3,0	7,9	12,1	55,1	2,6	8,1	12,3	26
V	253—255[b]	$C_{26}H_{15}NO_7S_2$	—	—	—	12,9	—	—	—	12,5	25
VII	205—207[c]	$C_{40}H_{31}NO_3S_4$ [e]	68,4	4,5	—	18,3	68,2	4,5	—	18,1	50
VIII	220—221[d]	$C_{26}H_{17}Br_2NO_3S_2$	50,0	2,8	—	50,6	50,6	2,8	—	10,5	90
IX	201—202[a]	$C_{28}H_{23}NO_3S_2$	69,3	4,8	—	13,0	69,3	4,8	—	13,2	30
XI	251—252[f]	$C_{12}H_5Br_2NO_3$ [g]	39,2	1,5	4,0	—	38,9	1,4	3,8	—	35
XIII	249—250[h]	$C_{21}H_{17}NO_3S$	69,2	4,7	—	8,8	69,5	4,8	—	8,8	82
XVI	263—265[i]	$C_{28}H_{23}NO_3S_2$	69,2	4,8	—	13,1	69,5	4,8	—	13,2	80

Note: [a]From isoamyl alcohol. [b]From 80% alcohol. [c]From n-butyl alcohol. [d]From n-butyl alcohol–isoamyl alcohol (1:1). [e]Found: Br 25.5%. Calculated: Br 26.0%. [f]From dimethyl formamide. [g]Found: Br 43.3%. Calculated: Br 43.1%. [h]From benzene or ethanol. [i]From ethanol.

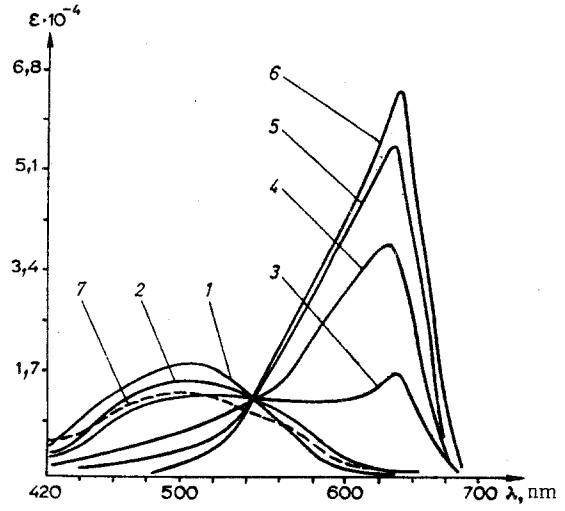

Fig. 1. Absorption spectra of alcohol solutions of II (1-6, c 10^{-4} M, layer thickness 5 mm): 1) pH 2.50; 2) pH 3.10; 3) pH 4.10; 4) pH 5.00; 5) pH 5.95; 6) pH 7.10; 7) IX (c $5 \cdot 10^{-5}$ M, layer thickness 5 mm).

In the present paper, the position of the two arylthio residues in the resorufin derivatives was established by a series of reactions, and the site of protonation of I during the described reaction was thereby determined.

It is known [1] that the bromine atom in bromophenoxazinones is only slightly labile and does not undergo nucleophilic substitution. Proceeding from this, one might have expected that the reaction of 2,4,6,8-tetrabromo-7-hydroxy-3-phenoxazinone (VI) [8] with thiocresol would give 1,9-di(tolylthio)-2,4,6,8-tetrabromoresorufin in the case of protonation of the molecule at the oxygen atom of the carbonyl group. However, it was established that all of the bromine atoms in tetrabromide VI are readily exchanged by arylthio residues to give 2,4,6,8-tetra(tolylthio)-resorufin (VII). The bromination of II leads to dibromo derivative VIII, the replacement of the bromine atoms in which by arylthio groups gives a compound that is completely identical to VII. This excludes activation of the 1 and 9 positions during the reaction. An additional confirmation is the formation of di(tolylthio) derivative IX during the reaction of 1,9-dimethylresorufin with thiocresol.

For the definitive establishment of the structure of di(tolylthio)resorufin II and, consequently, other di(arylthio) derivatives, one of the possible isomers – 2,8-di(tolylthio)-7-hydroxy-3-phenoxazinone – was obtained by independent synthesis. 2,4-Dihydroxybromobenzene (X) reacts with sodium nitrite in acidic media (a known method for the preparation of resorufin from resorcinol [9]) to give 2,8-dibromoresorufin (XI) (35% yield), which has a known orientation of the bromine atoms. On heating with thiocresol, the bromine atoms in this compound are smoothly exchanged by arylthio residues to give 2,8-di(tolylthio)-7-hydroxy-3-phenoxazinone, which is identical to II obtained directly from resorufin (see the above scheme). Thus the addition of thiophenols to resorufin proceeds at the 2 and 8 positions, and this in turn is evidence for protonation at the ring nitrogen atom.

The entry of two thiophenol residues into the resorufin molecule is probably associated with the possibility of quinoidization of the benzoid ring, inasmuch as only one thiophenol molecule is added in the 2 position (in analogy with 3-phenoxazinone) in the case of 7-ethoxy-3-phenoxazinone (XII).

Like phenoxazinones [10], II-IV, VI-VIII, and XI have two characteristic absorption bands in the near UV region (210-220 and 230-245 nm), the presence and position of which are independent of the pH of the

TABLE 2. Spectra and pK_a Values of Resorufin Derivatives (50% alcohol, 20°C)

Compound	λ_{max} of the non-ionized form, nm	lg ε	λ_{max} of the ionized form, nm	lg ε	pK_a
I	481	3,78	576	5,14	6,85±0,03
II	505	4,41	626	5,17	5,78±0,03
III	494	3,98	624	5,21	5,75±0,05
IV	488	4,50	600	4,95	4,88±0,02
VIII	505	4,33	640	5,22	4,86±0,02
XI	495	4,40	595	5,05	4,38±0,02
VII	488	4,30	634	4,83	3,41±0,01
VI	492	4,36	606	4,98	2,16±0,01

solution. These compounds have two maxima in the visible region, the position and intensity of which depend on the pH of the solution; this is, in all likelihood, associated with acid–base dissociation with respect to a hydroxyl group of the phenol type. There is only a long-wave band (620-650 nm) in the spectra at pH > 5 for II-IV and at pH > 3.5 for VI-VIII and XI, while there is only a short-wave band (482-506 nm) in the spectra for II-IV at pH < 4 for VI-VIII and XI at pH < 2. Both of the indicated bands are observed in the spectra of all of the compounds at intermediate pH values.

If the assumption of acid–base dissociation is correct, the long-wave absorption maximum that appears in the more alkaline region is related to the absorption of a symmetrical anion, while the short-wave absorption maximum that is observed in more acidic solutions is related to the absorption of the undissociated form of the dye.

To confirm this, we synthesized 2,8-di(tolylthio)resorufin ethyl ether (XVI) through the sodium (XIV) and silver (XV) salts of II and subjected it to spectroscopic investigation. The position of the absorption maximum of an alcohol solution of XVI is independent of the pH of the solution (it remains constant at ~ 500 nm) and is close to the position of the short-wave maximum of an aqueous alcohol solution of II (Fig. 1). Inasmuch as XVI is incapable of acid–base dissociation and exists only in the undissociated form, the short-wave absorption maximum of II (and of III, IV, VI-VIII, and XI) should be related to the absorption of a symmetrical anion. Thus the change in the spectra of solutions of the investigated compounds as the pH increases is due to splitting out of the proton of the hydroxyl group.

To determine the effect of the nature of the substituents in the 2, 4, 6, and 8 positions of resorufin on the strengths of the investigated acids, we studied the ionization constants (pK_a) of II-IV, VI-VIII, and XI by the spectrophotometric method in [11]. The weakest acid is unsubstituted resorufin (I, pK_a 6.85, Table 2). The introduction of both bromine atoms and arylthio residues leads to an increase in the acid ionization as compared with resorufin, i.e., the arylthio residues, like bromine atoms, display electron-acceptor properties with respect to resorufin.

The higher acidity of IV in the 2,8-disubstituted resorufin series is associated with the strong electron-acceptor effect of bromine atoms; the electron-acceptor effect of the arylthio residues is exerted to a lesser extent.

The value of the ionization constants of 2,8-di(arylthio) resorufin derivatives is in good agreement with the donor–acceptor properties of substitutents in the para position of the arylthio group. The introduction of a nitro group into the para position relative to the thio group reinforces the electron-acceptor effect of the arylthio residue and leads to an increase in the ionization of the IV molecule as compared with III; the electron-donor methyl group (II) induces only a slight reverse effect.

Bromine atoms have the greatest effect on the pK_a values in the investigated 2,4,6,8-tetrasubsituted resorufin derivatives and in 2,8-disubstituted resorufin derivatives. Thus, for example, tetrabromoresorufin (VI, pK_a 2.16, Table 2) is a strong acid, while the effect of the arylthio residues is less significant in the case of the tetrasubstituted derivatives.

EXPERIMENTAL

The electronic spectra of II-IV, VI-VIII, and XI were obtained with an SF-10 spectrophotometer. Solutions of II, III, VI-VIII, and XI ($1 \cdot 10^{-4}$ M in 96% ethanol) and of IV ($3 \cdot 10^{-5}$ M in 96% ethanol) were prepared for the spectrophotometric measurements.

The ionization constants were measured in 50% ethanol with an SF-4 spectrophotometer. Ammonia-acetate buffers were used as buffer solutions. The starting solution for VI was a 0.1 N solution in hydrochloric acid.

2,8-Di(p-tolylthio)resorufin (II). A) A 0.8 g (0.006 mole) sample of p-thiocresol and a few drops of concentrated hydrochloric acid were added to 1 g (0.005 mole) of resorufin in 20 ml of ethanol. The leuco compound began to form gradually, and the solution became green. In order to oxidize the leuco compound to dithio derivative II, the mixture was allowed to stand in the air at room temperature for 3 days with periodic stirring. The mixture became brown, and the crystals were removed by filtration and washed with alcohol to give 0.3 g of II (Table 1).

B) A total of 0.5 g (40%) of dithio derivative II was similarly obtained from 1 g (0.003 mole) of dibromoresorufin XI and 0.9 g (0.007 mole) of p-thiocresol.

2,8-Di(phenylthio)resorufin (III), 2,8-Di(p-nitrophenylthio)resorufin (IV), and 2,8-Di(o-carboxyphenylthio)resorufin (V). These compounds were similarly obtained by method A (Table 1).

2,4,6,8-Tetra(tolylthio)resorufin (VII, Table 1). A) A 0.5 g (0.004 mole) sample of p-thiocresol was added gradually with stirring and heating to 40-50° to 0.5 g (0.001 mole) of tetrabromo derivative VI in 200 ml of alcohol containing 10 drops of concentrated hydrochloric acid. The mixture was heated at 50° for another 2.5 h, after which it was heated to the boiling point and then allowed to stand at room temperature for 8 h. The precipitated crystals were removed by filtration and washed with alcohol to give 0.3 g of VII.

B) A total of 0.55 g of VII was similarly obtained from 0.5 g (0.0009 mole) of VIII and 0.3 g (0.0025 mole) of p-thiocresol.

2,8-Di(tolylthio)-4,6-dibromoresorufin (VIII). A 0.2 g (0.005 mole) sample of finely ground di(tolylthio)resorufin II was added to a solution of sodium ethoxide (0.3 g of sodium in 20 ml of alcohol), and the mixture was stirred at room temperature for 20 min. The precipitate was then removed by filtration and washed with alcohol. A total of 1 ml of bromine was added gradually with stirring to the thus obtained sodium salt of II in 15 ml of alcohol, after which the mixture was refluxed for 20 min and cooled. The precipitated red-brown crystals were removed by filtration and washed with alcohol and ether to give 0.25 g of VIII (Table 1).

1,9-Dimethyl-2,8-di(p-tolylthio)resorufin (IX, Table 1). This compound was obtained, by the method used to prepare tolylthio derivative II, from 0.5 g (0.002 mole) of 1,9-dimethylresorufin [12] and 0.5 g (0.004 mole) of p-thiocresol. The yield was 0.3 g.

2,8-Dibromoresorufin (XI, Table 1). An 11 g (0.058 mole) sample of 2,4-dihydroxybromobenzene [13] was added in portions with stirring at a mixture temperature of no higher than 60° to a solution of 11 g (0.16 mole) of sodium nitrite in 70 ml of concentrated sulfuric acid. The mixture was held at 95-100° for 4 h and then poured into 0.5 liter of water. The precipitate was removed by filtration and washed with water to neutrality to give 4 g of XI.

2-Tolylthio-7-ethoxy-3-phenoxazinone (XIII, Table 1). A 0.4 g (0.003 mole) sample of p-thiocresol and four drops of concentrated hydrochloric acid were added to a suspension of 0.5 g (0.0025 mole) of 7-ethoxy-3-phenoxazinone (XII) [14] in 15 ml of alcohol, and the mixture was stirred at room temperature until phenoxazinone XII had dissolved completely and the solution color had changed to light-green. It was then held at room temperature for 1 h, after which 10 ml of a freshly prepared 10% solution of ferric chloride was added, and the mixture was stirred. The resulting crystals were removed by filtration, washed with alcohol and ether, and dried to give 0.5 g of XIII.

2,8-Di(tolylthio)resorufin Sodium Salt (XIV). A 0.5 g (0.001 mole) sample of II was added to a solution of sodium ethoxide (from 20 ml of alcohol and 0.2 g of sodium), and the suspension was stirred at room temperature for 1.5 h. The green precipitate was removed by filtration and washed repeatedly with alcohol. The yield was quantitative. Found: S 13.2%. $C_{26}H_{18}NO_3S_2Na$. Calculated: S 13.3%.

2,8-Di(tolylthio)resorufin Silver Salt (XV). A 0.2 g sample of silver nitrate was added to a solution of 0.5 g (0.001 mole) of sodium salt XIV in 0.3 liter of water. The flocculent brown precipitate was removed by filtration, washed with alcohol, and dried.

2,8-Di(tolylthio)-7-ethoxy-3-phenoxazinone (XVI, Table 1). A 0.7 ml sample of ethyl iodide was added to a suspension of 0.4 g (0.007 mole) of silver salt XV in 10 ml of alcohol, and the r ixture was refluxed for 1 h. The hot reaction mixture was filtered, and the precipitated silver iodide was washed on the filter with hot alcohol. The filtrate was cooled, and the precipitate was removed by filtration, dried, and purified with a column filled with Al_2O_3 (elution with anhydrous chloroform). The orange fraction was evaporated to give 0.23 g (80%) of XVI.

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